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Induction kinetics of the nuclear proteins encoded by the early indoleacetic acid-inducible genes, *PS-IAA4/5* and *PS-IAA6*, in pea (*Pisum sativum* L.)

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Summary

The plant hormone indoleacetic acid (IAA) rapidly induces transcription of two genes, *PS-IAA4/5* and *PS-IAA6*, in pea that encode nuclear proteins. The proteins were expressed in *Escherichia coli* and polyclonal antibodies were raised. The proteins can neither be detected on immunoblots of pea extracts from IAA-treated epicotyls nor subcellularly localized by immunofluorescence, suggesting that they are of low abundance. However, they can be immunoprecipitated as ^{35}S-methionine-labeled proteins synthesized *in vivo* from control and IAA-treated tissue segments. Short-term time-course experiments indicate that the amounts of PS-IAA4/5 and PS-IAA6 proteins decrease dramatically in non-IAA-treated tissue. However, the hormone slightly increases the PS-IAA4/5 and significantly enhances the PS-IAA6 proteins compared with the initial amounts present in the tissue, despite a large induction of both mRNAs. A net increase in the amount of the *in vivo* synthesized PS-IAA6 is observed after a lag period of 30 min after addition of IAA. Little or no PS-IAA4/5 or PS-IAA6 protein is detected after 6 h of induction, even though *PS-IAA4/5* and *PS-IAA6* mRNAs remain detectable. Immunoprecipitation of *in vitro* translated polypeptides with mRNAs from various auxin-treated and untreated mono- and dicotyledonous plants reveals that similar proteins are encoded by constitutive or IAA-induced mRNAs. Phylogenetic analysis of 10 PS-IAA4-like proteins from various plant species reveals that the PS-IAA4 and PS-IAA6 proteins belong to different lineages, suggesting that they may have distinct functions. The data suggest that as a primary response to IAA plant tissues produce short-lived nuclear proteins whose synthesis is regulated at the transcriptional and post-transcriptional levels.

Introduction

Auxins, typified by the naturally occurring indoleacetic acid (IAA), are a group of plant hormones that exert a variety of effects on plant growth and development (Went and Thimann, 1937). Cell division, cell elongation, root development, apical dominance, vascular differentiation and photo- and gravitropisms are all believed to be controlled, at least in part, by auxin. It is now well established that auxin induces expression of specific genes in a number of plant organs, tissues or cultured cells, though the function of the encoded polypeptides, with few exceptions (Takahashi and Nagata, 1992; Takahashi *et al.*, 1989; Van der Zaal *et al.*, 1991), is unknown (Ainley *et al.*, 1988; Conner *et al.*, 1990; Hagen *et al.*, 1984; McClure *et al.*, 1989; Oeller *et al.*, 1993; Theologis *et al.*, 1985; Walker and Key, 1982; Yamamoto *et al.*, 1992a,1992b).

Expression of two genes in pea, *PS-IAA4/5* and *PS-IAA6*, is rapidly induced by IAA (within 5 min, Theologis *et al.*, 1985) and by protein synthesis inhibitors such as cycloheximide (CHX) (Theologis *et al.*, 1985). Auxin induces transcription of these genes; the hormone has no effect on the stability of the inducible mRNAs (Koshiba, Ballas and Theologis, in preparation). Their induction by cycloheximide, however, is due to both transcriptional activation as well as mRNA stabilization (Koshiba, Ballas and Theologis, in preparation). The encoded proteins, PS-IAA4 and PS-IAA6, share extensive amino acid sequence identity with eight auxin-regulated proteins from other plant species (Aux22 and Aux28, soybean (Ainley *et al.*, 1988); ARG3 and ARG4, mungbean (Yamamoto *et al.*, 1992a); AtAux2-11, AtAux2-27, IAA1 and IAA2, *Arabidopsis* (Abel *et al.*, 1994; Conner *et al.*, 1990)). Recently, it was demonstrated that the PS-IAA4-like proteins contain nuclear localization signals (Abel *et al.*, 1994; Abel and Theologis, submitted for publication). Pulse-chase and immunoprecipitation experiments have defined the half-lives ($t_{\frac{1}{2}}$) of the PS-IAA4/5 and PS-IAA6 to be 8 min and 6 min, respectively (Abel *et al.*, 1994). Their most prominent structural feature is the presence of a βαα motif similar to the β sheet DNA binding domain found in prokaryotic repressors of the Arc family (Abel *et al.*, 1994). Based on these data, it has been suggested that plant tissues express short-lived nuclear proteins as a primary response to IAA. The prospect arises that these proteins may have a regulatory function as activators or repressors of late genes responsible for mediating the various auxin responses (Abel *et al.*, 1994).

Our long-term effort is to elucidate their precise function during the early steps of auxin action. As a first step towards this goal, we expressed the *PS-IAA4* and *PS-IAA6* genes in *Escherichia coli* and produced rabbit polyclonal

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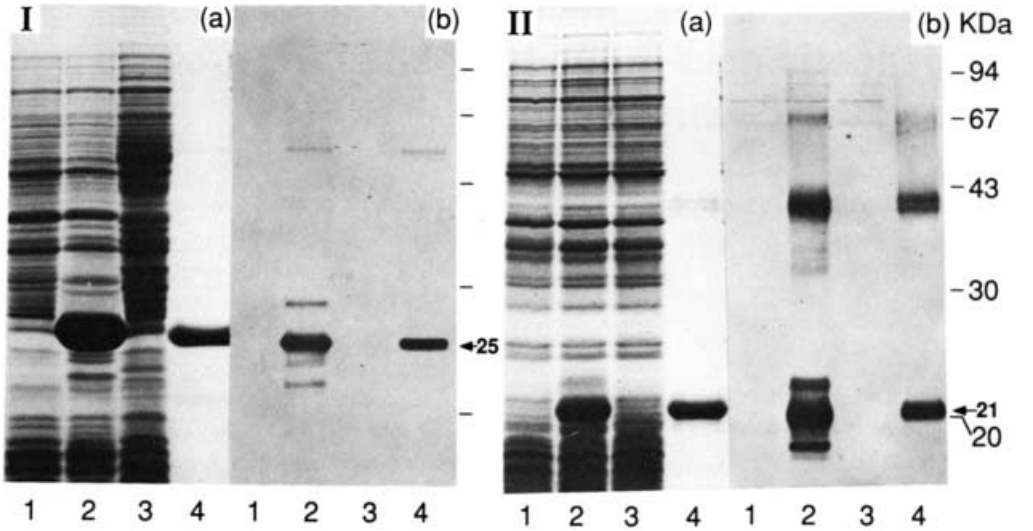

Figure 1. Expression of PS-IAA4 (panel I) and PS-IAA6 (panel II) proteins in *E. coli.*
The pIAA4/5 and pIAA6 cDNA inserts (Theologis *et al.*, 1985) were subcloned into a T7-based expression plasmid for *lac*-regulated expression in bacteria.
Panels I(a) and II(a) are portions of Coomassie blue-stained gels of bacterial extracts from IPTG-induced cells. The lanes are: 1, vector alone; 2, cDNA in sense orientation; 3, cDNA in antisense orientation; 4, purified recombinant protein. Each lane contains 100 μg protein except for lanes 4 which contain 5 μg purified bacterial protein.
Panels I(b) and II(b) are immunoblots of replica gels shown in I(a) and II(a), respectively, stained with affinity-purified anti-PS-IAA4 (panel I(b)) or anti-PSIAA6 (panel II(b)) antibodies. Each lane contains 100 ng protein except lanes 4 which contain 10 ng purified bacterial protein. The reference proteins are shown on the right: phosphorylase b, 94 kDa; BSA, 67; ovalbumin, 43; carbonic anhydrase 30; soybean trypsin inhibitor, 20. The arrows show the size of the recombinant proteins.

antibodies. The antibodies were used to determine the expression of *PS-IAA4*-like genes in various monocotyledenous and dicotyledonous plants by *in vitro* immunoprecipitation experiments. Induction kinetics of the PS-IAA4/5 and PS-IAA6 proteins after auxin treatment reveals that they are post-transcriptionally regulated.

Results

Expression of PS-IAA4 and PS-IAA6 proteins in E. coli *and antibody production*

The auxin-regulated cDNAs pIAA4/5 and pIAA6 (Oeller *et al.*, 1993; Theologis *et al.*, 1985) were inserted into a plasmid next to the phage T7 promoter for expression in *E. coli* (Oeller, 1992; Studier and Moffatt, 1986). Upon induction of cells carrying this plasmid with IPTG, large amounts of either PS-IAA4 (Figure 1, I(a), compare lane 2 with lane 1) or PS-IAA6 protein (Figure 1, II(a), compare lane 2 with lane 1) accumulate. *E. coli* strains expressing the cDNAs in antisense orientation do not accumulate any recombinant protein (Figure 1, compare lane 3 with lane 2 in I(a) for PS-IAA4, and II(a) for PS-IAA6). The proteins were solubilized with 8 M urea and purified to near homogeneity as described in Experimental procedures (Figure 1, compare lane 4 with lane 2 in I(a) for PS-IAA4, and II(a) for PS-IAA6). The purified proteins were used to raise high titer rabbit antisera. Affinity-purified antisera can specifically detect the recombinant proteins in crude bacterial extracts as well as the purified recombinant proteins. (Figure 1, lanes 2 and 4 in I(b) for PS-IAA4 and II(b) for PS-IAA6). Each antiserum can detect at least 0.2 ng of bacterially made protein by immunoblotting analysis (data not shown, see Oeller, 1992). However when 200 μg of total protein extract (corresponding to 20 mg of fresh weight of pea epicotyls) are immunoblotted, the antibodies fail to detect the PS-IAA4 and PS-IAA6 polypeptides. Similarly the antibodies fail to detect the proteins by immunocytochemical analysis, using thin sections of the third internode of etiolated pea seedlings (data not shown, see Oeller, 1992).

Immunoprecipitation of in vitro *and* in vivo *made PS-IAA4/5 and PS-IAA6 proteins*

To demonstrate that the antibodies can recognize the IAA-inducible polypeptides, immunoprecipitation experiments with *in vitro* translation products of mRNAs from control and IAA-treated tissue as well as with *in vivo* labeled proteins from IAA-treated and untreated tissue were carried out and the results are shown in Figure 2 (I is PS-IAA4/5, II is PS-IAA6). PS-IAA4 antibodies recognize several polypeptides which are encoded by auxin-inducible mRNAs

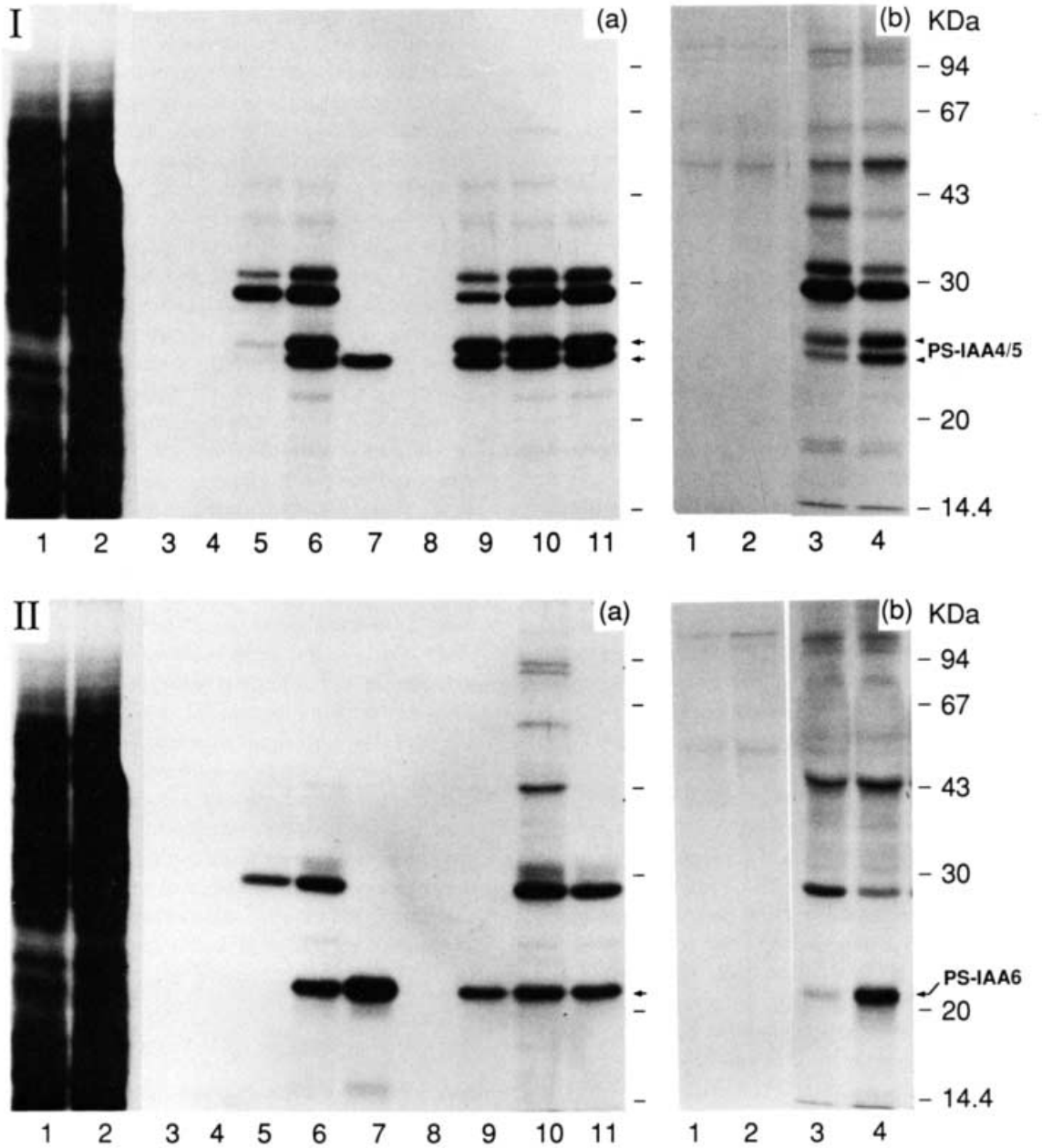

Figure 2. Immunoprecipitations of [^{35}S]methionine-labeled *in vitro* and *in vivo* made PS-IAA4 (panel I) and PS-IAA6 (panel II) proteins.
Panel I(a) shows an autoradiogram of immunoprecipitations with PS-IAA4 antibodies of *in vitro* translation products with poly(A)$^+$-RNA from control and IAA-treated (2 h) pea epicotyl tissue. The lanes are: 1 and 2, total translation products from control (1) and IAA-treated (2) tissue; 3 and 4, immunoprecipitations with pre-immune serum of the products shown in lanes 1 and 2; 5 and 6, as lanes 3 and 4 but with affinity-purified PS-IAA4 antibodies; 7, the product of *in vitro* synthesized *PS-IAA4* mRNA; 8–11, immunoprecipitations of IAA translational products in the presence of various competitor proteins: PS-IAA4 (8), PS-IAA6 (9), β-galactosidase (10), and BSA (11).
Panel I(b) shows an autoradiogram of immunoprecipitations using *in vivo* [^{35}S]methionine-labeled pea tissue from control (lanes 1 and 3) and auxin-treated (lanes 2 and 4) tissue using pre-immune serum (lanes 1 and 2) or affinity-purified PS-IAA4 antibodies (lanes 3 and 4).
Panels II(a) and II(b) are exactly as I(a) and I(b) but with PS-IAA6 antibodies. Also, lane 7 is the product of *in vitro* made *PS-IAA6* mRNA; lane 8, the competitor is PS-IAA6 protein and lane 9 the competitor is PS-IAA4 protein.
The data shown in panels I and II were obtained with the same samples. The reference proteins are as shown in the legend of Figure 1. For experimental details, see Experimental procedures.

(Figure 2, I(a), compare lane 6 with lane 5). A doublet of 25/26 kDa as well as an ~31 kDa polypeptide are strongly induced by IAA. The antibodies also immunoprecipitate a weakly IAA-inducible polypeptide of 29 kDa (Figure 2, I(a), compare lane 6 with lane 5). To identify which of the auxin-inducible polypeptides (shown in Figure 2, I(a), lane 6) are

encoded by the *PS-IAA4* mRNA, RNA was synthesized *in vitro* from a full-length cDNA, translated *in vitro* in a wheat germ lysate and immunoprecipitated with PS-IAA4 antibodies. The results show that the lower band of the 25/26 kDa doublet corresponds to the PS-IAA4 protein (Figure 2, I(a), compare lane 7 with lane 6). Furthermore, the results indicate that the upper band of the 25/26 kDa doublet is the translational product of a distinct mRNA and not a modified version of the 25 kDa PS-IAA4 polypeptide. The 26 kDa protein probably corresponds to the PS-IAA5 gene product whose mRNA structure has not yet been elucidated (Oeller *et al.*, 1993). The PS-IAA4 protein displays slightly aberrant mobility on SDS–PAGE, because its predicted molecular mass is 21 kDa (Table 1), but its apparent mass is 25 kDa (Figure 2, I(a), lane 7). The specificity of the immunoprecipitation was confirmed by competition with bacterially made PS-IAA4 protein (Figure 2, I(a), lane 8). Preimmune serum does not precipitate the auxin-inducible polypeptides (Figure 2, I(a), lanes 3 and 4). Structurally unrelated β-galactosidase (Figure 2, I(a), lane 10) and BSA (Figure 2, I(a), lane 11) do not compete with the immunoprecipitation. Excess PS-IAA6 protein competes with the immunoprecipitation of the 29 and 31 kDa polypeptides (Figure 2, I(a), lane 9), indicating that these proteins probably share some of the conserved domains common in PS-IAA4 and PS-IAA6 proteins (Abel *et al.*, 1994; Oeller *et al.*, 1993).

Immunoprecipitation of protein labeled *in vivo* with [^{35}S]methionine from control and IAA-treated pea epicotyl tissue also reveals an auxin-inducible doublet of 25/26 kDa that corresponds to the PS-IAA4/5 doublet detected in *in vitro* translation products (Figure 2, compare lanes 3 and 4 in I(b) with lane 6 in I(a)). Several other polypeptides that correspond to the *in vitro* translational products of 29 and 31 kDa whose synthesis appears to be repressed by IAA are also immunoprecipitated (Figure 2, compare lanes 3 and 4 in I(b) with lanes 5 and 6 in I(a)). The few additional immunoprecipitable products observed in the *in vivo* immunoprecipitations (Figure 2, compare lanes 3 and 4 in (b) with lanes 5 and 6 in (a) in I and II) may represent: (i) distantly related members of the multigene family encoding PS-IAA4/5-like proteins; or (ii) non-specific interactions(s) of the IgG with *in vivo* labeled proteins.

Similar experiments were carried out with the PS-IAA6 antibodies and are shown in Figure 2, II(a), and (b). Antibodies raised against the PS-IAA6 protein recognize a single polypeptide of 21 kDa derived from an auxin-inducible mRNA (Figure 2, II(a), compare lanes 5 with 6) as well as the weakly IAA-inducible 29 kDa polypeptide which is also immunoprecipitated with the PS-IAA4 antibody (Figure 2, II(a), compare lanes 5 and 6). PS-IAA6 mRNA synthesized *in vitro* specifies an immunoprecipitable polypeptide that co-migrates with the 21 kDa polypeptide encoded by an IAA-inducible mRNA (Figure 2, II(a), compare lane 7 with lane 6). The specificity of the immunoprecipitation was also confirmed by competition with bacterially made PS-IAA6 proteins (Figure 2, II(a), compare lane 8 with lane 6). Pre-immune serum also does not precipitate the auxin-inducible polypeptide (Figure 2, II(a), lanes 3 and 4), and unrelated proteins such as β-galactosidase and BSA do not compete with the immunoprecipitation (Figure 2, II(a), lanes 10 and 11, respectively). The additional bands seen when β-galactosidase is used as the competitor (Figure 2, II(a), lane 10) are attributed to non-specific protein aggregation during immunoprecipitation. Excess PS-IAA4 protein partially competes with the immunoprecipitation of the 21 kDa PS-IAA6 protein and fully competes with the precipitation of the 29 kDa polypeptide (Figure 2, II(a), compare lane 9 with lane 6) demonstrating that the conserved domains in PS-IAA4/5 and PS-IAA6 are probably also present in the 29 kDa protein (Abel *et al.*, 1994; Oeller *et al.*, 1993). We attribute the partial blockage of the PS-IAA6 immunoprecipitation by excess of PS-IAA4 protein to the presence of the conserved domains between these two proteins. Immunoprecipitation of *in vivo* labeled proteins with PS-IAA6 antibodies reveals an auxin-inducible protein of 21 kDa with the same mobility as the 21 kDa auxin-inducible polypeptide detected in *in vitro* made products (Figure 2, compare lanes 3 and 4 in II(b) with lanes 5 and 6 in II(a)). The correspondence between the PS-IAA4/5 and PS-IAA6 proteins made *in vitro* and *in vivo* demonstrates that these proteins are not extensively modified post-translationally, and that the antibodies recognize IAA-inducible polypeptides synthesized *in vivo*. Our failure to detect the proteins on immunoblots of pea extracts or in thin sections by immunofluorescence is probably due to their low abundance (Oeller *et al.*, 1993).

Kinetics of induction of PS-IAA4/5 and PS-IAA6 proteins

Previously, we showed that IAA transcriptionally activates the *PS-IAA4* and *PS-IAA6* genes with a lag period of 5–10 min (Koshiba, Ballas and Theologis, in preparation). To determine the induction kinetics of the proteins encoded by these genes, we used the PS-IAA4 and PS-IAA6 antisera to immunoprecipitate [^{35}S]methionine-labeled proteins from IAA-induced tissue at short time intervals after induction with IAA (Figure 3, I(b) for PS-IAA4/5 and I(d) for PS-IAA6). While RNA hybridization analysis shows a sixfold increase in PS-IAA4/5 mRNA and a 10-fold increase in PS-IAA6 mRNA after 30 min of IAA induction (Figure 3, I(a) and (c)), a slight decrease in PS-IAA4/5 and no change in PS-IAA6 proteins are observed at the same time of induction (Figure 3, I(b) and (d)). However, after 1 h of IAA treatment, when both mRNAs are induced almost 10-fold, a slight increase (10%) in the PS-IAA4/5 and a significant increase (two- to threefold) in PS-IAA6 proteins is observed com-

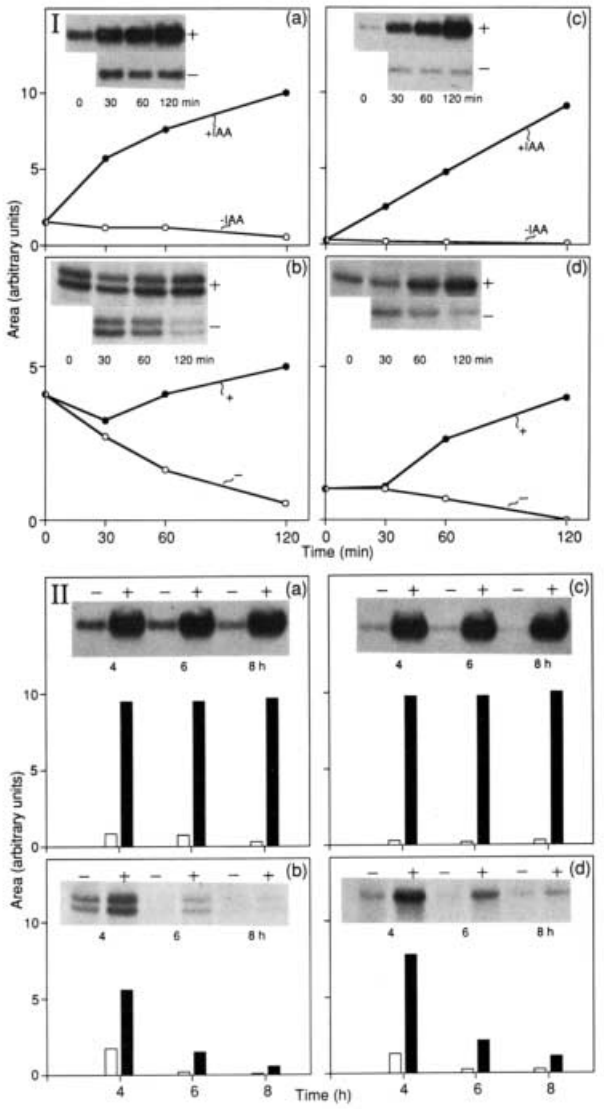

Figure 3. Induction kinetics of PS-IAA4/5 and PS-IAA6 proteins.
Panel I: short time-course experiments showing the rapidity of accumulation of the *PS-IAA4/5* and *PS-IAA6* mRNAs (a and c, respectively) and proteins (b and d, respectively) in pea epicotyl segments in the presence (+) or absence (-) of 20 μM IAA RNA.
Panel II: as in panel I, except over a long time course. RNA hybridization analysis with 20 μg total nucleic acids was carried out as described in Experimental procedures using ^{32}P-labeled pIAA4/5 or pIAA6 cDNA inserts (Oeller *et al.*, 1993). *In vivo* labeled proteins were immunoprecipitated from control and IAA-treated tissue as described in Experimental procedures. The incorporation of [^{35}S]methionine in c.p.m. per 0.8 g fresh weight of tissue into total tissue extract and TCA-precipitable material at various time intervals: total extract—4 h, 1.2×10^9; 6 h, 1.5×10^9; 8 h, 1.65×10^9; and TCA-precipitable—4 h, 0.33×10^9; 6 h, 0.5×10^9; 8 h, 0.66×10^9.

pared with the amount present in the initial tissue (t_0) (Figure 3, I(b) and (d)). After 2 h of treatment, both mRNAs and proteins continue to increase. The amounts of both proteins decrease dramatically in non-IAA-treated tissue and after 2 h they are five- to 10-fold lower than those in IAA-treated tissue (Figure 3, I(b) and (d)). A similar experiment was performed after long time intervals of induction (Figure 3, II(b) and (d)). The results indicate that both proteins can be detected after 4 h of IAA treatment, however after 6 h their level drops dramatically (Figure 3, II(b) and (d)) even though the *PS-IAA4/5* and *PS-IAA6* mRNAs are detectable and have reached their steady-state level (Figure 3, II(a) and (c)). The incorporation of [^{35}S]methionine is linear throughout the course of this long-term experiment. Only 40% of the [^{35}S]methionine has been incorporated into TCA-precipitable material after 8 h of treatment (see Figure 3, legend). If the synthesis of these proteins was occurring, they should have been detected by immunoprecipitation after 6 or 8 h. These results suggest that a post-transcriptional regulatory mechanism is operational on the *PS-IAA4/5* and *PS-IAA6* genes.

Identification of immunologically related proteins in other plant species

To determine whether other plant species express IAA-inducible mRNAs encoding related polypeptides, immunoprecipitation experiments with *in vitro* translation products and the antibodies to the pea proteins were carried out. The results are shown in Figure 4(a) PS-IAA4 and (b) PS-IAA6. PS-IAA4 antibodies immunoprecipitate at least 12 polypeptides from mRNA isolated from IAA-treated soybean hypocotyls, ranging in size from 17 to 40 kDa, 10 of which are IAA inducible (Figure 4(a), compare lane 4 with lane 3). The pattern of immunoprecipitable products from soybean mRNA is similar but not identical to that obtained with pea mRNA (Figure 4(a), compare lane 4 with lane 2). Similar immunoprecipitation detects at least six polypeptides in etiolated *Arabidopsis* seedlings (M_r 19–40 kDa) five of which are IAA inducible (Figure 4(a), compare lane 6 with lane 5). IAA-treated maize mesocotyls express at least seven mRNAs encoding proteins related to PS-IAA4 with M_r clustered around 30 kDa (Figure 4(a), compare lane 8 with lane 7). A similar diverse profile of polypeptides is recognized by the PS-IAA6 antibodies with mRNAs from the four plant species mentioned above (Figure 4b). The immunoprecipitation by both antibodies is specific because they can be competed using purified PS-IAA4 or PS-IAA6 protein (data not shown).

Phylogenetic analysis

Analysis of 10 PS-IAA4-like amino acid sequences from various plant species (Abel *et al.*, 1994) using the progressive alignment method developed by Doolittle and Feng (1990) yields the unrooted phylogenetic tree shown in Figure 5. The tree shows that the 10 auxin-regulated proteins fall into three distinct classes. Interestingly, although pea, soybean, and mungbean are closely related

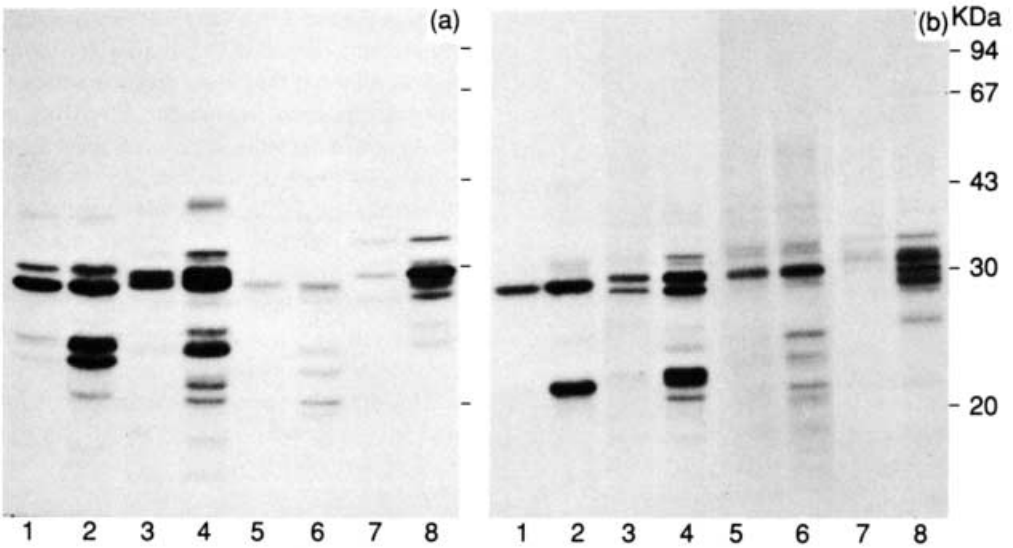

Figure 4. Immunoprecipitations of [^{35}S]methionine-labeled *in vitro* translation products with mRNAs from various plant species.
(a) Antibodies to PS-IAA4 protein were used to immunoprecipitate *in vitro* translation products with mRNA from control (lanes 1, 3, 5, 7) or auxin-treated tissue (lanes 2, 4, 6, 8). Lanes 1 and 2, etiolated pea epicotyls; lanes 3 and 4, etiolated soybean hypocotyls; lanes 5 and 6, etiolated *Arabidopsis* hypocotyls; lanes 7 and 8, etiolated maize mesocotyls.
(b) Exactly as (a) but antibodies to PS-IAA6 protein were used for immunoprecipitations. The same samples were used to obtain the data shown in (a) and (b). The reference proteins are as shown in the legend of Figure 1.

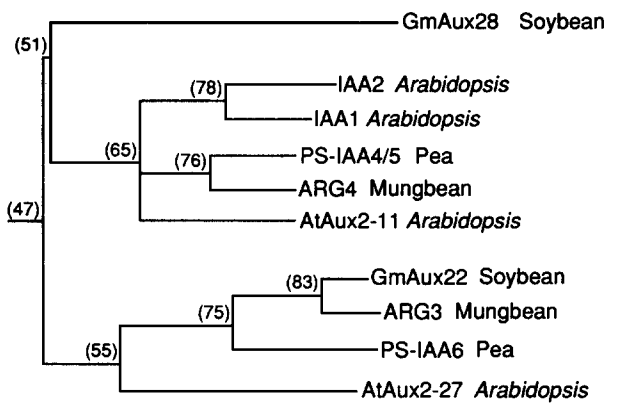

Figure 5. Amino acid sequence-based phylogeny of the auxin-regulated polypeptides.
The unrooted phylogenetic tree was constructed from the alignment shown in Figure 1 in Abel *et al.* (1994) using the progressive alignment method of Doolittle and Feng (1990). The horizontal distances are drawn to scale and the numbers in parentheses are the averaged percentage identities of all pairwise comparisons of the sequences positioned to the right. Sources of the sequences are (a) pea (*Pisum sativum*) (Oeller *et al.*, 1993); (b) soybean (*Glycine max*) (Ainley *et al.*, 1988); (c) mung bean (*Vigna radiata*) (Yamamoto *et al.*, 1992b); (d) *Arabidopsis thaliana* (*AtAux2-11* and *AtAux2-27* (Conner *et al.*, 1990), *IAA1* and *IAA2* (Abel *et al.*, 1994)).

evolutionarily by being members of the Leguminoseae family, the GmAux28 protein of soybean has diverged from the other two lineages which include proteins from *Arabidopsis thaliana*, a member of the Crucifereae family. The possibility exists that the proteins of each lineage perform similar but distinct functions. As more members of the multigene family from each species are isolated, a more complex tree will be obtained indicating the diversity of the proteins utilized for mediating the auxin responses.

Discussion

Properties of the PS-IAA4 and PS-IAA6 proteins

The polypeptides encoded by the *PS-IAA4* and *PS-IAA6* mRNAs are similar in size and isoelectric point (Table 1), and their overall hydropathy profiles are similar (data not shown). Neither protein contains a putative signal peptide or N-linked glycosylation sites (von Heijne, 1986; Kornfeld and Kornfeld, 1985). Both proteins are not grossly modified *in vivo* since the mobilities of the *in vitro* translated products are indistinguishable from those of the proteins synthesized *in vivo* (Figure 2).

Alignment of their amino acid sequences reveals the presence of four conserved domains, I, II, III and IV, ranging in size from 9 to 43 amino acids (Abel *et al.*, 1994; Oeller *et al.*, 1993). Both proteins share similar physical properties (Table 1) and extensive amino acid sequence identity with eight other proteins encoded by early auxin-regulated genes in soybean, mungbean and *Arabidopsis* (Abel *et al.*, 1994). All these proteins contain the four conserved domains found in PS-IAA4 and PS-IAA6 (Abel *et al.*, 1994). The existence of similar genes in other plant species is documented by the already cloned genes, but the extent

Table 1. Properties of the polypeptides encoded by various auxin-regulated genes from various plant species

Protein	No. of amino acids	Predicted molecular mass (Da)	Isoelectric point	Plant species	Reference
PS-IAA4	189	21 036	6.4	Pea	Abel *et al.* (1994)
PS-IAA6	179	20 330	6.9	Pea	Abel *et al.* (1994)
GmAux22	195	21 478	7.2	Soybean	Ainley *et al.* (1988)
GmAux28	243	26 910	5.5	Soybean	Ainley *et al.* (1988)
ARG3	194	21 514	7.2	Mungbean	Yamamoto *et al.* (1992b)
ARG4	196	22 049	8.2	Mungbean	Yamamoto *et al.* (1992b)
AtAux2-11[a]	186	20 990	5.6	*Arabidopsis*	Conner *et al.* (1990)
AtAux2-27[a]	174	19 595	8.3	*Arabidopsis*	Conner *et al.* (1990)
IAA1	168	19 062	8.2	*Arabidopsis*	Abel *et al.* (1994)
IAA2	174	19 910	5.2	*Arabidopsis*	Abel *et al.* (1994)

[a]The properties have been estimated from the amino acid sequence deduced from genomic sequences.

of the multigene family is striking as revealed by the immunoprecipitation of *in vitro* translation products shown in Figure 4. The PS-IAA4 and PS-IAA6 antibodies recognize a similar group of polypeptides, some of which are encoded by auxin-inducible mRNAs, while others are constitutively expressed in pea, soybean, *Arabidopsis* and maize (Figure 4). It is of a great interest that 14 IAA-inducible PS-IAA4-like genes have been cloned from *Arabidopsis* and all contain the four conserved domains (Abel and Theologis, unpublished). Recent experimental evidence indicates that the PS-IAA4-like proteins are nuclear localized and contain a βαα motif similar to the β-sheet DNA-binding domain found in prokaryotic repressors of the Arc family (Abel *et al.*, 1994).

The PS-IAA4 and PS-IAA6 proteins are of low abundance

Our antibodies cannot detect the proteins on immunoblots of pea extracts from IAA-treated tissue, nor can they be used to localize them subcellularly by immunofluorescence (Oeller, 1992). The antibodies, however, can specifically immunoprecipitate IAA-inducible PS-IAA4/5 and PS-IAA6 proteins from *in vivo* [^{35}S]methionine-labeled proteins (Figure 2). The immunoprecipitated proteins shown in Figure 2 (I(b) and II(b), lanes 3 and 4) represent all the immunoreactive material from 0.5 g fresh weight of tissue (~25 segments) labeled with 0.5 mCi [^{35}S]methionine for 2 h and require several days of exposure to be visualized by fluorography. We estimate that these nuclear proteins represent no more than 0.0001% of the total protein in pea epicotyl tissue. This calculation refers to the inability to detect these proteins on immunoblots and is based on the amount of total protein loaded on the gel (200 μg) and the minimum amount of protein that can be detected by the antibodies (0.2 ng). From these results, we also estimate that approximately 3000 molecules of PS-IAA4 or PS-IAA6 protein are present in each cell assuming that every cell expresses these genes. This was based on the average cell size (~10 pl; 25 μm × 20 μm × 20 μm), the assumption that one pea tissue segment (~20 μl) contains 2×10^6 cells, and the fact that 0.2 ng of a 21 kDa protein corresponds to $\sim 6 \times 10^9$ molecules. The low abundance of the proteins can be attributed to their short $t_{\frac{1}{2}}$, which is 8 min for PS-IAA4 and 6 min for PS-IAA6 (Abel *et al.*, 1994), among the shortest known half-lives for eukaryotic proteins. Such short half-lives are characteristic of regulatory molecules (Gottesman and Maurizi, 1992; Varshavsky, 1992). The *Drosophila ftz* and yeast MATα2 transcription factors, involved in development and differentiation, have half-lives of 5–10 min (Edgar *et al.*, 1987; Hochstrasser and Varshavsky, 1990; Kellerman *et al.*, 1990). The proto-oncogene products *myb*, *myc* and *fos*, which are transcription factors involved in growth control, are also known to be rapidly turned over, with half-lives of 25–60 min (Klempnauer *et al.*, 1986; Lüscher and Eisenman, 1988; Mitchell *et al.*, 1985). Enzymes such as ornithine decarboxylase ($t_{\frac{1}{2}}$=10 min (Isomaa *et al.*, 1983)) and 1-aminocyclopropane-1-carboxylic acid (ACC) synthase ($t_{\frac{1}{2}}$=58 min (Kim and Yang, 1992)), which catalyze rate-limiting steps of biosynthetic pathways, also generally have short half-lives.

The mechanism of PS-IAA4 and PS-IAA6 degradation has not yet been investigated. We note, however, that neither protein contains a typical 'PEST' region associated with rapid turnover (Rogers *et al.*, 1986). If the N-end rule applies to these proteins, the expected post-translational modification exposing the N-terminus of the mature protein would likely result in a minor mobility change on an SDS gel that would probably not be detected in our experiments (Varshavsky, 1992). We have not used anti-ubiquitin antibodies to determine if these proteins are targeted by this mechanism, and we cannot detect transient higher molecular weight species indicative of ubiquitination (see Figure 2). The low abundance of the proteins may make the detection of ubiquitinated forms difficult.

Kinetics of protein induction

Studies of transcriptionally inducible genes have shown that the period required to attain new levels of individual mRNAs and proteins is related to their unique half-lives (Hargrove *et al.*, 1991). The minimum period required to achieve a new level is directly proportional to product half-lives because rates of decay control the ratio between the rate of synthesis and the concentration of gene products at steady state (Hargrove *et al.*, 1991). Based on this generally accepted model for the kinetics of induction processes, half-maximal induction should be detected after the equivalent of one $t_{\frac{1}{2}}$, assuming the degradation rate is not changed. The $t_{\frac{1}{2}}$s of PS-IAA4/5 and PS-IAA6 protein have been determined to be 8 min and 6 min, respectively (Abel *et al.*, 1994). According to the kinetic model, half-maximal induction of these proteins should be detected after 8 min and 6 min, respectively. The data of Figure 4, however, indicate a 30 min lag period before an increase in protein levels is detected, while the corresponding mRNAs start to accumulate after a lag period of 5–10 min (Theologis *et al.*, 1985). This discrepancy is easily resolved by the kinetic model that states that when the rate of transcription changes, the time required to achieve a new level of the encoded protein is a function of the half-lives of all intermediates (i.e. mRNA). The $t_{\frac{1}{2}}$s of *PS-IAA4/5* and *PS-IAA6* mRNAs have been experimentally determined to be 60 min and 72 min, respectively (Koshiba, Ballas and Theologis, in preparation). A quantitative model has been developed that predicts the rate of protein accumulation as a function of mRNA concentration (Hargrove *et al.*, 1991) and the $t_{\frac{1}{2}}$s of mRNAs and proteins. Computer programs are available that provide solutions to the mathematical equation that describes this model. Using the microcomputer program STELLA® II (Hargrove, 1993; Hargrove *et al.*, 1993), we were able to simulate the kinetics of induction of PS-IAA4/5 and PS-IAA6 proteins using the experimentally determined $t_{\frac{1}{2}}$s of their mRNAs and proteins (Figure 6). The simulated curve for PS-IAA6 (Figure 6b) quantitatively fits the experimental data shown in Figure 3 (I(c) and (d)), whereas that for PS-IAA4/5 (Figure 6a) qualitatively fits the experimental data shown in Figure 3 (I(a) and (b)). This discrepancy may be due to incomplete knowledge of the simulation parameters (e.g. mRNA translatability). The amount of both proteins drops during the first 20 min of the simulation; by 30 min the experimentally determined level of PS-IAA4/5 is lower than the initial level (compare Figure 3 I(b) with that of Figure 6a) whereas the level of PS-IAA6 is almost the same as its initial level (compare Figure 3 I(d) with Figure 6b). The decrease in the amount of the proteins in the first 20–30 min is attributed to the more rapid turnover of the proteins (PS-IAA4/5, $t_{\frac{1}{2}}$ 8 min; PS-IAA6, $t_{\frac{1}{2}}$ 6 min; Abel *et al.*, 1994) compared with that of the corresponding mRNAs (*PS-IAA4/5*, $t_{\frac{1}{2}}$ 60 min; *PS-IAA6*, $t_{\frac{1}{2}}$ 72 min; Koshiba and Theologis, unpublished) (Yagil, 1975). The simulated data of Figure 6 also indicate that the net increase of both proteins is moderate whereas their corresponding mRNAs increase more dramatically, and agree with the experimental evidence so far obtained (Theologis *et al.*, 1985; and Figure 3).

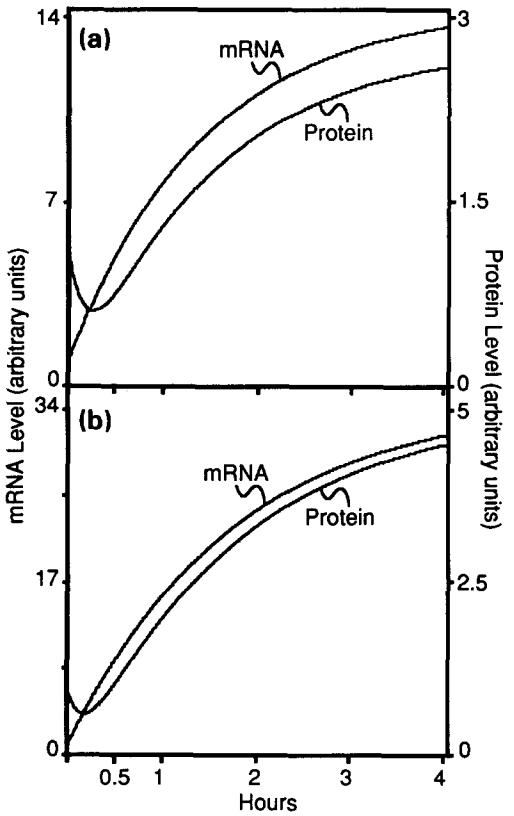

Figure 6. Predicted time course for induction of PS-IAA4/5 (a) and PS-IAA6 (b) mRNAs and proteins using the STELLA® II microcomputer program for kinetic modeling of gene expression (Hargrove, 1993; Hargrove *et al.*, 1993). Theoretical accumulation curves for PS-IAA4/5 and PS-IAA6 proteins and mRNAs were computed, based on the known $t_{\frac{1}{2}}$s of PS-IAA4/5 and PS-IAA6 proteins (8 min and 6 min, respectively; Abel *et al.* (1994)) and mRNAs (60 min and 72 min, respectively; Koshiba, Ballas and Theologis, in preparation). The simulation assumes the rate of mRNA synthesis due to transcriptional activation by IAA (Koshiba, Ballas and Theologis, in preparation) increases 10-fold for PS-IAA4/5 and 20-fold for PS-IAA6 and that the initial level of each mRNA and protein in the uninduced state is 1.

After a long period of IAA induction, little or no PS-IAA4/5 and PS-IAA6 protein can be detected by immunoprecipitation (Figure 3, II(b) for PS-IAA4/5 and II(d) for PS-IAA6). In contrast, the PS-IAA4/5 and PS-IAA6 mRNAs remain easily detectable ([Figure 3, II(a) and (c)). Protein synthesis is still occurring in tissues treated for 6–12 h with IAA and poly(A)$^+$ RNA isolated from 12 h treated tissue is translationally active *in vitro* (Oeller, 1992). The results reported here suggest that a translational control mechanism is operational after 4 h of IAA treatment. Perhaps PS-IAA4/5 and PS-IA6 proteins regulate their own synthesis

by interacting with *PS-IAA4/5* and *PS-IAA6* mRNAs, blocking their translation (Mitchell *et al.*, 1985).

The proteins do not accumulate before the onset of cell elongation induced by IAA, and disappear while elongation is still occurring (Jacobs and Ray, 1976; and Figure 3). The proteins are expressed in a short window of time relative to the elongation response, which continues for at least 24 h (Jacobs and Ray, 1976; Katsumi, 1963). This pattern of expression is similar to that of growth regulators in vertebrate systems such as the proto-oncogene *c-fos* which is detectable for only a few hours upon serum stimulation of a quiescent cell culture. During this time it is able to establish conditions that lead to cell proliferation (Kruijer *et al.*, 1984; Müller *et al.*, 1984).

Though the absolute level of induction of these proteins (three- to fivefold) is low, it can be enough to change the growth state of the cell dramatically, and in fact is reminiscent of several well-known regulators in bacteria and yeast. The induction of phage lambda from its prophage state is known to require only a fivefold change in intracellular repressor concentration (Ptashne, 1986). Similarly, in yeast, a sixfold change in Gal4 concentration causes a 40-fold change in expression of Gal1, a structural gene required for galactose utilization (Griggs and Johnston, 1991). Such subtle changes of concentration having switch-like properties are due to cooperativity, a common feature of regulatory mechanisms in biological systems. The precise regulatory function of these short-lived nuclear proteins has not yet been defined. We have proposed that they may be part of a cascade response involved in auxin-mediated differentiation (Abel *et al.*, 1994) similar to the ecdysone-mediated cascade of gene expression during insect development (Ashburner, 1990; Segraves and Hogness, 1990). The precise function of the PS-IAA4-like proteins observed in various plant species will be eventually determined by further biochemical characterization and functional inactivation using reverse genetic approaches.

Experimental procedures

Plant material

Seven-day-old etiolated pea seedlings (*Pisum sativum* cv. Alaska) were grown in vermiculite, and 8-mm-long epicotyl segments from the third internode of each seedling were treated with 20 μM IAA as previously described (Theologis *et al.*, 1985). Etiolated soybean (*Glycine max* cv. Prize) and corn (*Zea mays* cv. Funk) seedlings were also grown in vermiculite in total darkness for 5 days; 1 cm segments from the elongating region of the hypocotyl (soybean) or from the mesocotyl (corn) were used for hormone treatment. Etiolated *Arabidopsis* seedlings (*Arabidopsis thaliana* ecotype Columbia) were grown for 5 days in complete darkness on solid 0.5 × MS medium (Murashige and Skoog, 1962) and harvested by shaving off the seedlings about 1 cm above the surface of the plate. Auxin treatment of soybean, corn and *Arabidopsis* tissues was performed using 20 μM IAA for 2 h (Theologis *et al.*, 1985). After incubation, the tissue was frozen in liquid nitrogen and stored at –70°C.

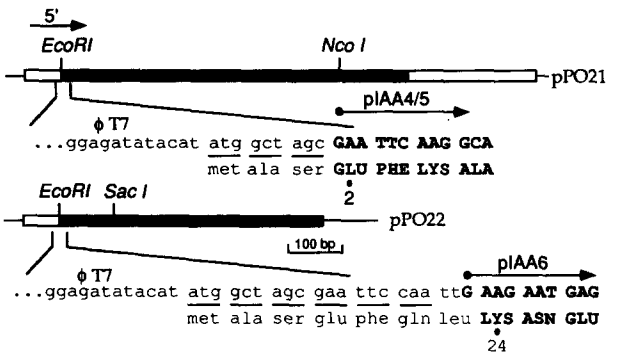

Figure 7. Partial restriction maps of pPO21 and pPO22 plasmids used for expression of the PS-IAA4/5 (pPO21) and PS-IAA6 (pPO22) proteins in *E. coli* (Studier and Moffatt, 1986).
See Experimental procedures for details of their construction. The nucleic acid and amino acid sequences at the fusion junction of the ϕT7 promoter of pPO9 and the modified pIAA4/5 and pIAA6 cDNA inserts are shown below. The nucleotides and amino acids corresponding to the ϕT7 gene are shown in lower case letters while those of pIAA4/5 and pIAA6 cDNAs are shown in upper case letters.

Expression of PS-IAA4 and PS-IAA6 in E. coli, *protein purification, and antibody production*

The T7 RNA polymerase-based expression vector, pET-3C (Studier and Moffatt, 1986), was modified to allow expression of cDNAs cloned as *Eco*RI fragments by introducing a unique *Eco*RI site 9 bp downstream from the translation initiation codon giving rise to the pPO9 vector (Rottmann *et al.*, 1991). An open reading frame cloned into the *Eco*RI site of pPO9 in the correct register will give rise to a polypeptide with three additional amino acid residues at its amino terminus. The pIAA4/5 plasmid (Oeller *et al.*, 1993; Theologis *et al.*, 1985) was digested with *Eco*RI and the 810 bp insert, containing the entire coding region except the initiating methionine, was subcloned into the *Eco*RI site of pPO9 giving rise to pPO21 (see Figure 7). The plasmid pPO20 was also constructed containing the cDNA insert in antisense orientation. Plasmid pIAA4/5 selects two highly similar mRNAs as determined by hybrid selected translation experiments (Theologis *et al.*, 1985) thus its notation. However, the protein encoded by the pIAA4/5 cDNA insert corresponds to *PS-IAA4* mRNA (Oeller *et al.*, 1993). The structure of the mRNA encoding the polypeptide PS-IAA5 has not yet been determined. Consequently, the antibodies raised with the pIAA4/5 insert correspond to PS-IAA4 protein. The plasmid pIAA6 (Oeller *et al.*, 1993) was digested with *Eco*RI, the 761 bp cDNA isolated and treated briefly with exonuclease *BAL*31 (Sambrook *et al.*, 1989). The ends were made blunt with Klenow, *Eco*RI linkers (5'-GGAATTCC-3') were added, and the fragment was subcloned into the *Eco*RI site of pUC18 giving rise to pPO19. The pPO19 cDNA insert was subcloned into the *Eco*RI site of pPO9 giving rise to pPO22 (sense, Figure 7) and pPO23 (antisense) expression plasmids. The protein fusion produced from the pPO22 plasmid consists of seven NH_2-terminal amino acids derived from the *Eco*RI linker and the pPO9 vector, one hundred and fifty amino acids representing the PS-IAA6 protein (amino acids 24–173) and seven COOH-terminal amino acids derived from the pPO9 vector.

The pPO20, pPO21, pPO22, and pPO23 plasmids were introduced

into the expression competent host BL21(DE3) (Studier and Moffatt, 1986). Expression of PS-IAA4 and PS-IAA6 was carried out at 37°C for 3 h in the presence of 2 mM IPTG, as described by Studier and Moffat (1986). Induced BL21(DE3) cells harboring either pPO21 or pPO22 were collected by centrifugation for 10 min at 4°C. The pellet was washed with 20 mM sodium phosphate buffer, pH 7.0, 5 mM EDTA, and resuspended in 1/50 of the original culture volume using the same buffer. The cell suspension was subjected to repeated cycles (five to six) of freeze, thaw and sonication, using a Heat Systems Ultrasonics Inc. sonicator (model W-385) fitted with a standard tapered microtip. The lysate was centrifuged at 100 000 ***g*** for 30 min at 4°C and the supernatant was dialyzed against 20 mM sodium phosphate buffer (pH 7.0), 5 mM EDTA for 48 h at 4°C, changing the buffer every 8 h. Upon completion of dialysis, a precipitate was visible which was found to be almost pure protein. The precipitated protein was dissolved in 20 mM Tris–HCl (pH 8.0), 100 mM NaCl, 1 mM dithiothreitol, 8 M urea and dialyzed extensively against the same buffer without urea. The suspension was centrifuged at 16 000 ***g*** for 10 min at 4°C and the pellet discarded. The supernatant was found to contain pure PS-IAA4 or PS-IAA6 protein. Pure protein (30–40 mg) was obtained from 1 l of induced culture. Antibodies to recombinant proteins were produced in New Zealand White rabbits (Harlow and Lane, 1988). The antibodies were purified by affinity chromatography using PS-IAA4 or PS-IAA6 proteins immobilized on CNBr-activated Sepharose-4B (Harlow and Lane, 1988). These antibodies have been previously used for determining the $t_{\frac{1}{2}}$ of PS-IAA4/5 and PS-IAA6 proteins (Abel *et al.*, 1994). Immunoblotting was performed according to Burnette (1981), and the antigen–antibody complexes were visualized with alkaline phosphatase conjugated on a goat anti-rabbit IgG (Promega).

In vivo *labeling and immunoprecipitation*

For *in vivo* labeling experiments, the pea epicotyl tissue was manually abraded, using a paste of 5 μm aluminum oxide, cut to 8 mm segments and treated with or without IAA in the presence of 1 mCi [^{35}S]methionine (Trans label, ICN, sp. act. >1000 Ci mmol^{-1}) using buffers previously described (Theologis *et al.*, 1985).

(i) Short time course. Forty abraded segments (~0.8 g fresh weight per sample) were incubated in depletion medium (Theologis *et al.*, 1985) for 0.5 h at 25°C and subsequently transferred to the same medium containing 1 mCi [^{35}S]methionine (Trans label, ICN, sp. act. >1000 Ci mmol^{-1}) for an additional 2 h. Subsequently, the hormone treatment was initiated with 20 μM IAA in the presence of [^{35}S]methionine, and tissue samples were taken at the indicated times and processed for immunoprecipitation.

(ii) Long time course. Tissue samples were depleted for 0.5 h, treated for 2 h with or without 20 μM IAA in incubation buffer (Theologis *et al.*, 1985) and, subsequently, 1 mCi [^{35}S]methionine was added to each sample and, tissue was removed at the indicated times and processed for immunoprecipitation.

Labeled tissue was frozen in liquid N_2, ground to a fine powder using a mortar and pestle, and lyophilized. Protein was extracted with 1 ml boiling extraction buffer (100 mM sodium phosphate buffer pH 7.0, 140 mM β-mercaptoethanol, 1% (w/v) SDS) to inactivate endogenous proteases (Vierstra and Quail, 1982). Immunoprecipitations were performed with 2×10^6 c.p.m. (*in vitro*) and 1×10^8 c.p.m. (*in vivo*) of acid-precipitable, [^{35}S]methionine-labeled proteins using pre-immune and affinity-purified immune antiserum as described (Giudice *et al.*, 1979) except the trichloroacetic acid (TCA) precipitation was omitted. The homogenate was centrifuged at 100 000 ***g*** for 20 min at 4°C and the supernatant was diluted 10-fold with 1% Triton X-100, 20 mM sodium phosphate buffer, pH 7.0, 5 mM EDTA. Affinity-purified antibodies were added corresponding to 100 μl of crude antiserum. The antibodies were incubated with agitation for 10–15 h at 4°C and protein A-Sepharose (25 μl packed volume) was added and incubated for 2 h at 4°C. The antigen–antibody–protein A complex was collected by centrifugation and washed as described (Giudice *et al.*, 1979) except that the wash buffer was adjusted to 0.4 M LiCl for the first two of the five wash steps (Giudice *et al.*, 1979). The immunoprecipitates were eluted into 35 μl boiling Laemmli loading buffer and resolved on a 12% SDS–polyacrylamide gel (Laemmli, 1970) using 0.5× buffer in the separating gel. Gels were stained with Coomassie brilliant blue, destained, and soaked in 1 M sodium salicylate (Chamberlain, 1979) before drying. Fluorograms were quantified using an LKB scanning densitometer.

Synthesis of full length PS-IAA4 and PS-IAA6 RNAs in vitro

PS-IAA4. Plasmid pIAA4.1 containing an almost full-length PS-IAA4 cDNA (Oeller *et al.*, 1993) was digested with *Eco*RI giving rise to two *Eco*RI fragments, one 346 bp containing the 5′ end, and another 870 bp containing the 3′ end of the cDNA. The 346 bp *Eco*RI fragment was digested with restriction endonuclease *Mae*III and the digestion products were ligated to an adaptor, formed by annealing two oligomers, T7451 (5′ TGTAATACGACTCACTCTCGGGATCCACAAGT 3′) and T7452 (5′ GTAACACTTGTGGATCCCTATAGTGAGTCGTATTACA 3′), and pUC19 digested with *Hin*cII and *Eco*RI. These ligation products were used to transform *E. coli* DH5α (Sambrook *et al.*, 1989). The T7451/T7452 adaptor contains a phage T7 RNA polymerase promoter (nucleotides 3–19 of T7451), a *Bam*HI recognition site (nucleotides 21–26 of T7451), the first 6 nucleotides of the IAA4/5 mRNA (nucleotides 27–32 of T7451) and a *Mae*III 5′ overhang (nucleotides 1–5 of T7452). Numerous transformants were isolated and screened by restriction enzyme digests to determine whether they contained the correct insert. A plasmid, designated pPO33, was isolated and its authenticity was verified by DNA sequence analysis (Sambrook et al., 1989). Subsequently, the 870 bp fragment (3′ end) was subcloned into the *Eco*RI site of pPO33 giving rise to pPO36. This plasmid contains a bacteriophage T7 RNA polymerase promoter followed by a *Bam*HI site followed by a full-length PS-IAA4 cDNA including a poly(A) tail 53 nt long.

PS-IAA6. Plasmid pIAA6 (Oeller *et al.*, 1993) containing a partial PS-IAA6 cDNA was digested with *Sac*I and *Eco*RI and the 526 bp fragment, containing the 3′ portion of the cDNA, was gel purified by adsorption to NA45 paper (Schleicher & Schuell). The 5′ end of the cDNA was constructed by PCR using the genomic subclone p*Bam-Hin*dIII (Oeller *et al.*, 1993) as a template and the amplimers IA6S (5′ GGCGAGCTCCATCCATGCTAACC 3′) and IA6BI (5′ CCGGGATCCTACATGTTCAAAAAATATTCC 3′). The amplified fragment (257 bp) corresponds to the 5′ end of the mature PS-IAA6 mRNA (from the transcription start site to the *Sac*I site). The 5′ fragment was digested with *Sac*I/*Bam*HI and ligated with the previously isolated 3′ 526 bp *Sac*I/*Eco*RI fragment into *Bam*HI/*Eco*RI-digested pPO33. The resulting plasmid, designated pPO57, contains the bacteriophage T7 RNA polymerase promoter followed by a *Bam*HI site followed by a full-length IAA6 cDNA including a poly(A) tail 8 nt long.

Plasmids pPO36 or pPO57 were digested with *Eco*RI (pPO36

requires partial digestion) and the linearized plasmids were gel purified by adsorption on glass powder. Two micrograms of each plasmid were incubated in 40 mM Tris (pH 8), 8 mM $MgCl_2$, 5 mM DTT, 4 mM spermidine, 1.25 mM dNTPs, 40 U RNasin (Promega) and 60 units of T7 RNA polymerase (Promega) for 60 min at 37°C. The DNA templates were digested with 4 units of RNase-free DNase for 15 min at 37°C, and the RNA was extracted with phenol and ethanol precipitated. The RNAs synthesized migrate as a single species on an agarose gel.

mRNA isolation and in vitro *translation*

Total RNA and poly(A)$^+$ RNA were isolated as previously described (Theologis *et al.*, 1985). RNA blots were prepared and hybridized using standard methods (Sambrook et al., 1989). Poly(A)$^+$ RNA or *in vitro* synthesized RNA was translated *in vitro* using a wheat germ extract from Amersham in the presence of [^{35}S]methionine (sp. act. 800 Ci mmol^{-1}) as recommended by the manufacturer. *In vitro* RNA synthesis was performed using plasmids pPO36 and pPO57 as templates containing full-length PS-IAA4 and PS-IAA6 cDNAs, respectively (Malone *et al.*, 1989).

Phylogenetic analysis

The amino acid sequences of 10 polypeptides encoded by auxin-regulated genes were aligned to generate a phylogenetic tree by using Parsimony After Progressive Alignment (PAPA) programs (Doolittle and Feng, 1990) provided by Dr Russell F. Doolittle (U.C. San Diego) and compiled on a SUN-UNIX computer.

Biochemicals

Murashige and Skoog (1962) salt mixture was from Gibco. Ultrapure SDS and urea were from Schwartz-Mann. Oligo(dT)-cellulose, $m_7G(5')ppp(5')G$, NTPs, and protein molecular weight standards were from Pharmacia. Nitrocellulose (BA-85) was from Schleicher & Schuell. All other chemicals were from Sigma and Aldrich. Seeds were from Musser Seed (pea), Burpee (soybean), Pioneer Hybred (corn) and Guhy's Specialty Nursery (*Arabidopsis*).

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References

Abel, S., Oeller, P.W. and Theologis, A. (1994) Early auxin-induced genes encode short-lived nuclear proteins. *Proc. Natl Acad. Sci. USA*, **91**, 326–330.

Ainley, W.M., Walker, J.C., Nagao, R. and Key, J.L. (1988) Sequence and characterization of two auxin-regulated genes from soybean. *J. Biol. Chem.* **263**, 10 658–10 666.

Ashburner, M. (1990) Puffs, genes, and hormones revisited. *Cell*, **61**, 1–3.

Burnette, N.W. (1981) Western blotting: electrophoretic transfer of proteins from sodium dodecylsulfate polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* **112**, 195- 203.

Chamberlain, J.P. (1979) Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. *Anal. Biochem.* **98**, 132–135.

Conner, T.W., Goekjian, V.H., LaFayette, P.R. and Key, J.L. (1990) Structure and expression of two auxin-inducible genes from *Arabidopsis*. *Plant Mol. Biol.* **15**, 623–632.

Doolittle, R.F. and Feng, D.F. (1990) Nearest neighbor procedure for relating progressively aligned amino acid sequences. *Methods Enzymol.* **183**, 659–669.

Edgar, B.A., Odell, G.M. and Schubiger, G. (1987) Cytoarchitecture and the patterning of *fushi tarazu* expression in the *Drosophila* blastoderm. *Genes Devel.* **1**, 1226–1237.

Giudice, L.C., Waxdall, M.J. and Weintraub, B.D. (1979) Comparison of bovine and mouse pituitary glycoprotein hormone pre-α subunits synthesized *in vitro*. *Proc. Natl Acad. Sci. USA*, **76**, 4798–4802.

Gottesman, S. and Maurizi, M.R. (1992) Regulation by proteolysis: Energy-dependent proteases and their targets. *Microbiol. Rev.* **56**, 592–621.

Griggs, D.W. and Johnston, M. (1991) Regulated expression of the GAL4 activator gene in yeast provides a sensitive switch for glucose repression. *Proc. Natl Acad. Sci. USA*, **88**, 8597–8601.

Hagen, G., Kleinschmidt, A. and Guilfoyle, T. (1984) Auxin-regulated gene expression in intact soybean hypocotyl and excised hypocotyl sections. *Planta*, **162**, 147–153.

Hargrove, J.L. (1993) Microcomputer-assisted kinetic modeling of mammalian gene expression. *FASEB J.* **7**, 1163–1170.

Hargrove, J.L., Hulsey, M.G. and Beale, E.G. (1991) The kinetics of mammalian gene expression. *BioEssays*, **13**, 667–674.

Hargrove, J.L., Hulsey, M.G. and Summers, A.O. (1993) From genotype to phenotype: computer-based modeling of gene expression with STELLA II. *BioTechniques*, **15**, 1096–1101.

Harlow, D. and Lane, E. (1988) *Antibodies*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Von Heijne, G. (1986) A new method for predicting signal sequence cleavage sites. *Nucl. Acids Res.* **14**, 4683–4690.

Hochstrasser, M. and Varshavsky, A. (1990) In vivo degradation of a transcriptional regulator: the yeast α2 repressor. *Cell*, **61**, 697–708.

Isomaa, V.V., Pajunen, A.E.I., Bardin, C.W. and Janne, O. (1983) Ornithine decarboxylase in mouse kidney. *J. Biol. Chem.* **258**, 6735–6740.

Jacobs, M. and Ray, P. (1976) Rapid auxin-induced decrease in free space pH and its relationship to auxin-induced growth in maize and pea. *Plant Physiol.* **58**, 203–209.

Katsumi, M. (1963) Physiological effects of kinetin. Effect of kinetin on the elongation, water uptake and oxygen uptake of etiolated pea stem sections. *Physiol. Plant.* **16**, 66–72.

Kellerman, K.A., Mattson, D.M. and Duncan, I. (1990) Mutations affecting the stability of the *fushi tarazu* protein of *Drosophila*. *Genes Devel.* **4**, 1936–1950.

Kim, W.T. and Yang, S.F. (1992) Turnover of 1-aminocyclopropane-1-carboxylic acid synthase protein in wounded tomato fruit tissue. *Plant Physiol.* **100**, 1126–1131.

Klempnauer, K.H., Bonifer, C. and Sippel, A.E. (1986) Identification and characterization of the protein encoded by the human *c-myb* proto-oncogene. *EMBO J.* **5**, 1903–1911.

Kornfeld, R. and Kornfeld, S. (1985) Assembly of asparagine-linked oligosaccharides. *Ann. Rev. Biochem.* **54**, 631–664.

Kruijer, W., Cooper, J.A., Hunter, T. and Verma, I.M. (1984) Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. *Nature*, **312**, 711–716.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

Lüscher, B. and Eisenman, R.N. (1988) c-myc and c-myb protein degradation: Effect of metabolic inhibitors and heat shock. *Mol. Cell. Biol.* **8**, 2504–2512.

Malone, R.W., Felgner, P.L. and Verma, I.M. (1989) Cationic liposome-mediated RNA transfection. *Proc. Natl Acad. Sci. USA*, **86**, 6077–6081.

McClure, B.A., Hagen, G., Brown, C.S., Gee, M.A. and Guilfoyle, T.J. (1989) Transcription, organization and sequence of an auxin-regulated gene cluster in soybean. *Plant Cell*, **1**, 229-239.

Mitchell, R.L., Zokas, L., Schreiber, R.D. and Verma, I.M. (1985) Rapid induction of the expression of proto-oncogene *fos* during human monocyte differentiation. *Cell*, **40**, 209–217.

Müller, R., Bravo, R., Burckhardt, J. and Curran, T. (1984) Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. *Nature*, **312**, 716–720.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15**, 473–497.

Oeller, P.W. (1992) *Molecular aspects of plant hormone action*, Ph.D. Dissertation. St. Louis, MO: Washington University.

Oeller, P.W., Keller, J.A., Parks, J.E., Silbert, J.E. and Theologis, A. (1993) Structural characterization of the early indoleacetic acid-inducible genes, *PS-IAA4/5* and *PS-IAA6*, of pea (*Pisum sativum* L.). *J. Mol. Biol.* **233**, 789–798.

Ptashne, M. (1986) *In A Genetic Switch*, Cambridge, MA/Palo Alto, CA: Cell Press/Blackwell Scientific Publications, pp. 27–31.

Rogers, S., Wells, R. and Rechsteiner, M. (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science*, **234**, 364–368.

Rottmann, W.E., Peter, G.F., Oeller, P.W., *et al.* (1991) 1-Aminocyclopropane-1-carboxylate synthase in tomato is encoded by a multigene family whose transcription is induced during fruit and floral senescence. *J. Mol. Biol.* **222**, 937–961.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Segraves, W.A. and Hogness, D.S. (1990) The E75 ecdysone-inducible gene responsible for the E75B early puff in Drosophila encodes two new members of the steroid receptor superfamily. *Genes Devel.* **4**, 204–219.

Studier, F.W. and Moffatt, B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113–130.

Takahashi, Y. and Nagata, T. (1992) *parB*: an auxin-regulated gene encoding glutathione S-transferase. *Proc. Natl Acad. Sci. USA*, **89**, 56–59.

Takahashi, Y., Kuroda, H., Tanaka, T., Machida, Y., Takebe, I. and Nagata, T. (1989) Isolation of an auxin-regulated gene cDNA expressed during the transition from Go to S phase in tobacco mesophyll protoplasts. *Proc. Natl Acad. Sci. USA*, **86**, 9279–9283.

Theologis, A., Huynh, T.V. and Davis, R.W. (1985) Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. *J. Mol. Biol.* **183**, 53–68.

Van Der Zaal, E.J., Droog, F.N.J., Boot, C.J.M., Hensgens, L.A.M., Hoge, J.H.C., Schilperoort, R A. and Libbenga, K.R. (1991) Promoters of auxin-induced genes from tobacco can lead to auxin-inducible and root tip-specific expression. *Plant Mol. Biol.* **16**, 983–998.

Varshavsky, A. (1992) The N-End rule. *Cell*, **69**, 725–735.

Velazquez, L., Fellous, M., Stark, G.R. and Pellegrini, S. (1992) A protein tyrosine kinase in the interferon α/β signalling pathway. *Cell*, **70**, 313–322.

Vierstra, R.D. and Quail, P.H. (1982) Native phytochrome: inhibition of proteolysis yields a homogeneous monomer of 124 kiloDaltons from *Avena*. *Proc. Natl Acad. Sci. USA*, **79**, 5272–5276.

Walker, J.C. and Key, J.L. (1982) Isolation of cloned cDNAs to auxin-responsive poly(A)$^+$ RNAs of elongating soybean hypocotyl. *Proc. Natl Acad. Sci. USA*, **79**, 7185–7189.

Went, F.W. and Thimann, K.V. (1937) *Phytohormones*. New York: Macmillan.

Yagil, G. (1975) Quantitative aspects of protein induction. *Curr. Topics Cell Reg.* **9**, 183–286.

Yamamoto, K.T., Mori, H. and Imaseki, H. (1992a) Novel mRNA sequences induced by indole-3-acetic acid in sections of elongating hypocotyls of mung bean (*Vigna radiata*). *Plant Cell Physiol.* **33**, 13–20.

Yamamoto, K.T., Mori, H. and Imaseki, H. (1992b) cDNA cloning of indole-3 acetic acid-regulated genes: Aux22 and SAUR from mung bean (*Vigna radiata*) hypocotyl tissue. *Plant Cell Physiol.* **33**, 93–97.